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ANOPHELES AND SEA WATER,

WITH OBSERVATIONS ON THE INFLUENCE OF SALINITY ON THE DEVELOPMENT OF AMERICAN SPECIES.

By T. H. D. Griffitts, Epidemiologist, United States Public Health Service.

In interpreting the geographical distribution of malarial fevers and the occurrence of insect carriers of the disease, a more intelligent appreciation of the problem can be gained from a study of the chemical content of water as well as biological environments. The relation between the amount and kind of chemical in water and the presence of mosquitoes in it is not as well understood as the purely biological relationships. The influence of the presence of salt in varying degrees in bodies of water, on mosquito life, has not been given the consideration it deserves. Two points seem especially worthy of further investigation: first, whether *A. quadrimaculatus* ever thrives in brackish water, as the results of the work of Smith and others would seem to indicate; and, second, whether there is epidemiological evidence tending to prove that malaria is transmitted by anophelines developed in brackish waters. In this connection it has been stated by investigators in Malaya that there is evidence that *A. rossii* developed in brackish water is a vector of malaria whereas, when developed in fresh water, it is doubtfully so.

Various writers have recorded instances in which mosquito larvæ have been observed to thrive in sea water. Some references are given below.

Smith (1904) states: "The species of Anopheles will breed wherever they can find water. There is no limit of size or kind of pool, and except that they do not occur in really foul liquids, they may be found wherever any other mosquito can breed. I have seen them in my experiment pails, in rain barrels, in gutters, in lot pools, in swamps, in the salt marshes, in woodland pools, in ditches, at the edges of running streams, in ponds, and even in springs." The same writer elsewhere says of *A. punctipennis*: "On the whole, it breeds most abundantly in clean water along the edges of pond or swamp areas or in the eddies of shallow streams." Concerning *A. quadrimaculatus* he says: "The breeding places are similar, but this form also occurs in brackish water on the salt marshes, hence has a somewhat wider range and adds the positive danger of disease to the disadvantages of an undrained marsh."

Dutton (1903), on his malaria expedition to Gambia, collected tidal water on several occasions and found it to contain Anopheles larvæ, which later developed into adult mosquitoes (*A. costalis*). Water collected in tidal pools in drains near the sluice gates, supporting larvæ of *A. costalis*, contained 1,038.5 parts of chlorine per 100,000 parts.

De Vogel (1907) found that the investigations of several Italian workers have negatived the idea that Anopheles can multiply in pure sea water. According to Peronne the maximum proportion of sodium chloride in the water which Anopheles larvæ can stand is 1.87 per cent, and according to Vivante, 1.75 per cent. De Vogel, having made some elaborate studies in regard to malaria at Samarang, Java, found as early as 1902 that a species of Anopheles was breeding in a certain pool containing 2.8 per cent of chloride of sodium. As an example of anophelines occurring in saline pools, he cites a marine station on the island of Onrust, which is 2,000 meters from mainland and contains no fresh water, but which had to be abandoned because of the ravages of malaria. This was believed to be due to Anopheles breeding in the sea water on the island. Malaria was a serious problem in the Karimon Islands before the sea-water pools were dried. Anopheles larvæ were found on the island of Grand Marimon, in pools containing not less than 3 per cent of sodium chloride. In a pool of water at Samarang, which had a surface of 20 to 30 square meters, a depth of 10 to 30 centimeters, and a percentage of 2.88 of sodium chloride, Anopheles larvæ were swarming, whereas Culex larvæ were not found. The author draws the following conclusions:

1. There are species of Anopheles which can live very well in sea water.
2. These mosquitoes lay eggs which develop even in sea water which has been evaporated to half its original quantity.
3. These larvæ in the gradually evaporating pools of sea water can stand an evaporation of the water to one-third of its bulk, but do not appear to transform to adults if the concentration be greater than this.
4. The larvæ coming from eggs laid in sea water in high concentration can accomplish their entire metamorphoses in almost the normal time. This is true even when the water has such concentration that the development of larvæ originally hatching in unconcentrated sea water would be retarded by this salt water.

In the opinion of de Vogel, the bad malarial reputation which the coral islands of the East Indies suffer is explained by his investigations, since many cases of malaria are observed along the coast during the dry season, when the rivers and fresh water streams are dried up. Villages near the sea, in the middle of tidal pools, have during a period of ten years an average mortality of from 1 to 4 per cent each year. In villages farther away from the sea, where the ponds have been abandoned or neglected and the sea water is, therefore, isolated, there is a mortality which varies from 8 to 10 per cent each year. During the dry season the pools in these regions have a proportion of sea salt equal to that of the ocean. In this dry season the death rate is greatest, and this is exclusively due to the Anopheles breeding in the sea-water ponds. The difference in the malarial rate is not due to any change in the character of the water itself. When the pools were tide-water pools, fish and other life had access and kept the mosquito larvæ in check, whereas in the subsequent isolated pools the Anopheles larvæ could develop unhindered.

Foley and Yvernault (1908) found in Algeria that an Anopheles, *Pyretophorus chaudoyei*, was able to breed in very saline waters. The same they note as being true of *Anopheles vagus* found in the Dutch East Indies.

Banks (1908) reports that *Myzomia ludlowii*, Theob., a species of Anopheles which probably transmits the subtertian malaria parasite, breeds in the Philippines, both in salt and fresh water, and altitude up to 1,500 meters has no appreciable effect upon its development. He thinks that it was originally a fresh-water species only, and has adapted itself to a marine life. *Nyssorhynchus stephensi*, an Indian species and a malaria carrier, has also been found breeding in salt water.

Clerc (1909) experimented with larvæ of *Anopheles maculipennis*, and found that the larvæ placed in water with 44 to 46 grams of salt to the liter of water would die if very young, but the older larvæ developed and produced imagoes.

Gholap (1910) discovered the larvæ of *N. stephensi* in ponds containing sea water at Colaba near Bombay. There were millions of the larvæ present in these water collections.

Willcocks (1910) records finding in Egypt, larvæ of a species of *Pyretophorus* (*P. cleopatræ*) flourishing in large numbers in brackish waters containing from 2.56 to 3.25 per cent of common salt. Even 1 per cent proved fatal to the larvæ of the common Egyptian Anopheles, *Cellia pharoensis*.

Le Prince and Orenstein (1916) give the following account of mosquito larvæ in salt and brackish waters:

"At Cristobal, Beach Island, in the Rio Grande Valley, and at Gatun, Anopheles larvæ have been found in brackish and salt water. In the first three locations the propagation areas were affected directly by tide water. At Cristobal, in that part of the tidal flats covered by high tides and by excessive tides, larvæ were found to be numerous wherever clearings were made and leaves remained in the water. Clumps of plant stems afforded hiding places to the larvæ of *A. albimanus* and *A. tarsimaculata*, even when small fish were present. Invariably larvæ were most numerous where the fallen leaves were most plentiful. In the swampy area in the Rio Grande Valley the percentage of salt water varied with the tide and rainfall. *Anopheles albimanus* was the prevailing species. The deep water contained many mangrove trees and drift from upstream, whereas the more shallow was well covered with grass, dead leaves, and plants that thrive in brackish water in the Tropics. Larvæ could always be found in untreated portions of this area where there were sufficient hiding places. The area was about a mile in length.

"The water in the grass around the edges of the newly formed lagoon (a flat depression north of Gatun Dam) remained nearly fresh, and no salt could be tasted along shore. In places where tall grass grew, salt was not perceptible to taste 600 feet from the shore line. In wading out from the shore the water was tasted every few yards, and it was noted that young Culex and Anopheles larvæ appeared with the first indication of brackishness. In going farther from the shore, as the water became more brackish the Anopheles larvæ found were more numerous and more mature. When the water became salty enough to be decidedly disagreeable to taste, Anopheles larvæ were most numerous. They were more numerous per unit of area than had been noted anywhere on the Isthmus during the previous nine years of antimosquito work. The absence of Anopheles and the scarcity of Culex larvæ in the wet zone not affected by salt water was unique. Tests made at many points along the shore established the fact. The condition was so uniform that by wading slowly from shore to shore with eyes closed, and testing by taste alone, we were able to reach the infested zone and secure larvæ in collecting cups. Small larvæ-destroying fish were quite numerous, but larvæ of Anopheles and Culex were so plentiful in the salty water that it was impossible for the fish to make any reduction. The species present was chiefly *A. tarsimaculata,* although *A. albimanus* and Culex were very plentiful. The production area continued in existence for several months, and frequent analyses of the water for sea-water content were made. In places where the larvæ were very numerous the water contained 60 per cent or more of sea water, and at times above 80 per cent."

Barber (1918), referring to *Anopheles rossii* states: "The comparatively high percentage of infections observed by me in the brackish water type of var. *indefinitus,* would bring this form under suspicion, although sporozoites apparently are not readily formed. Epidemiological evidence in the coast regions of the Federated Malay States is at fault, since this type of *rossii* is there so commonly associated with *ludlowi* and *umbrosus,* both known carriers. * * * The occurrence of this type (Giles) in brackish water, the ordinary breeding place of *A. ludlowi* in Malaya, is noteworthy, since the larvæ of type Giles and that of *ludlowi* appear identical."

Howard, Dyar, and Knab state: "In America several species of Anopheles have been found to breed in brackish water, but none of them exclusively so. *Anopheles crucians* has been found to breed in brackish water in New Jersey and Louisiana, and we have already mentioned that Smith has found larvæ of *A. quadrimaculatus* in New Jersey under similar circumstances. It is worthy of note in this connection that *A. crucians* seems to thrive best in the vicinity of tidewater and to occur much less abundantly inland."

With reference to the effect of salinity upon *mosquitoes other than Anopheles,* Chidester and Patterson (1916) reach the following conclusions:

The degree of salinity of the pools of the salt marshes on the New Jersey coast is about 7 or 8 per cent, but may be subject to greater fluctuations. Two series of experiments were carried out to determine the effect of marked changes in salinity on the larvæ of *Aëdes sollicitans* and *Aëdes cantator*. In the first series, larvæ were transferred from pools to water varying from a 13 per cent salinity to distilled water. In the second series, larvæ were placed in solutions varying from 16 to 35 per cent salinity. None was able to survive in the 22 per cent or higher concentration for more than two days. Further examination of pools showed that in one case, larvæ of *Aëdes sollicitans* were living in water with a 22 per cent salinity, at a temperature of 64° F., whereas none was present in a pool a short distance away, where the salinity was 24 per cent and the temperature 67° F. Other records show that *Aëdes sollicitans* was able to withstand a higher degree of salinity than *Aëdes cantator*. The distribution of various species of mosquitoes over the salt marshes appears to be dependent to a certain extent on the amount of salt present in the water; this factor may also influence the development of the eggs.

OBSERVATIONS ON THREE AMERICAN SPECIES.

In connection with the control of malaria in extra-cantonment zones located in "tidewater" country, and in the course of other malaria investigations, extending, in all, over a period of four years, the writer has had occasion to note something of the relative breeding habits of *A. quadrimaculatus, A. crucians,* and *A. punctipennis* in relation to various strengths of sea water. The observations herein noted were made in certain areas bordering upon the Chesapeake Bay, Hampton Roads (or its tidal tributaries), and the Atlantic Ocean at Virginia Beach, Va.

Anopheles crucians.—(1) Near Langley Field, Hampton, Va., in September, 1917, a large degree of infestation of *A. crucians* in barns was found. The production area was found to be a salt marsh three-fourths of a mile away. Subsequent and repeated examinations revealed *A. crucians* breeding (and producing) generally in the marsh in water showing a salinity of 10,088. (Hampton Roads at Newport News showed a salinity of 10,146). *Aëdes sollicitans* was also producing profusely in the same water. The *A. crucians* larvæ were usually found in the salt grass, *Distichlis spicata,* the smaller and finer of the principal marsh grasses found in this locality. The larger salt-marsh grass, *Spartina glabra,* grows more abundantly where there is more tidal action, and *A. crucians* was found propagating in this grass only where its growth was much retarded or where it was dead.

No other species of Anopheles bred in this marsh, although a fresh-water pond in the vicinity was producing *A. quadrimaculatus*, and the fresh-water streams near by were producing *A. punctipennis*.

(2) At Virginia Beach, Va., Lake Rudee, originally a tidal stream and salt marsh, is intermittently formed by a deposit of sand at the beach, which blocks the outlet and impounds salt water. With the addition of fresh water from small streams and from rainfall, the water of this lake becomes less saline. At different times from August to October, 1919, the grassy borders of this lake were found to be harboring *A. crucians* in great numbers, larvæ of all sizes, or pupæ, were taken at every selective dip. This lake, the salinity of which had undergone no appreciable change during several weeks, conformed to Carter's classification of a "complete" breeding place, i. e., the eggs deposited here hatched and the succeeding stages of larvæ or pupæ through to the imago stage were completed in the same water. The salinometer reading here was 10,068 (the salinity of the ocean water nearby was 10,196). This lake then had 34.6 per cent of sea water at this point. Here was a body of diluted sea water covering approximately five acres, producing *A. crucians*, a malaria vector, in sufficient numbers to be of decided sanitary importance to the community. Extending to near Lake Rudee and separated therefrom by a strip of elevated land only a few hundred feet wide, was Lake Holly, which, with very rare exceptions, was fresh water. During the time of observations, the highest salinity of Lake Holly was 10,003. This lake was producing *A. quadrimaculatus* in great numbers. Was not a striking contrast afforded by these lakes in the matter of selective breeding places of the two species? Lake Rudee, containing 34.6 per cent sea water, had an Anopheles production of 100 per cent *A. crucians;* Lake Holly, practically fresh (1.5 per cent sea water), an Anopheles production of 100 per cent *A. quadrimaculatus*. The two breeding places were separated by a distance of less than 600 feet. In a stable on this strip of land, on a day in early September, there were collected 67 specimens of *A. crucians* and 85 specimens of *A. quadrimaculatus*.

(3) At West Point, Va., York River showed a salinity of 10,110. A pond had been formed by dumping rubbish across a salt marsh, for the purpose of extending a street. The fill was very loose and porous. No culvert had been installed. The main tidal stream was blocked, and at high tide additional salt water entered the pond through the fill. However, the level of the pond varied little between tides. The salinity at the lower portion of the pond was 10,077, or 70 per cent York River water. This degree of salinity would be approximately 50 per cent of that of sea water along this section of the Atlantic coast. *A. crucians* was breeding and producing profusely in this water.

Two hundred feet away, in a different portion of the same body of water, at a point where, owing to the large amount of seepage and the lack of mixing of the waters by wave action, the salinity was only 10,003, *A. quadrimaculatus* was producing freely.

(4) Experimental: Larvæ of all sizes and pupæ of *A. crucians* collected from obstructed ditches (salinity 10,088) in the marsh of Back River, near Langley Field, September 9, 1920, were placed in sea water, salinity 10,160, on September 10, and were not unfavorably affected by the transfer. All but three, which were of the first molt when placed in the sea water, had developed into imagoes within 12 days. Even the smallest of the larvæ developed as well in the sea water as those in the fresh water control.

Anopheles quadrimaculatus.—(1) As has been stated, Lake Holly, at Virginia Beach, is practically always fresh. Some years ago a flume was constructed connecting this lake with the ocean, for the purpose of admitting sea water at high tide. In its condition at the time of these observations, salt water came into the lake only at times of very high storm tide; so that not even sufficient salinity was attained to prevent the production of *A. quadrimaculatus.* In many parts of the lake, prior to the successful control operations in 1920, this species was found breeding freely. The highest salinity found at any time was 10,003, or approximately 1.5 per cent sea water at this place. No species of Anopheles other than *A. quadrimaculatus* was found breeding in this lake at any time. *A. quadrimaculatus* was breeding profusely in another fresh-water pond one-half mile distant, and *A. crucians* bred in the saline Lake Rudee a few hundred feet away.

(2) The small body of impounded water at West Point, previously noted, covered an area approximately 3 acres in extent, in which a growth of reeds and salt grass (*Distichlis spicata*) still remained. As noted, the lower, saline portion of the pond was producing *A. crucians.* On the opposite side of the pond, 200 feet away, where seepage outcrop rendered the water fresh, only *A. quadrimaculatus* was breeding. The salinometer reading here was 10,003. The land rises abruptly from the narrow valley, and the protection of the steep sides of the valley in which this part of the pond lies, and the vegetation in the water, served to prevent wave action and the mixing of the waters. Consequently, the water on this side of the pond remained almost entirely fresh.

(3) A pond at the upper end of a salt marsh near Newport News produced *A. quadrimaculatus* in great numbers in 1917. This pond was one of those notorious products of road construction which are found in tidewater countries, where roads are built across salt marshes and culverts are set at too high an elveation or are inadequate in capacity, resulting in the impounding of fresh or more or less saline water above the fill. In this particular case, at times of

storm tides, salt water was admitted to the fresh water of the pond. However, the salinity was never so strong or so continuous as to kill the luxuriant growth of cat-tails that occupied practically all of the 2 acres of the pond, except a small portion near the effluent culvert. On one occasion at high tide the lower portion of the pond showed a salinity of 10,076. At this time, breeding was under control. The question naturally arises as to whether or not the larvæ and pupæ of *A. quadrimaculatus* may resist a relatively high degree of salinity intermittently. The determination of this point has a practical bearing on malaria control and could probably be demonstrated readily by experiments.

(4) In 1918, near Lee Hall and Camp Eustis, Va., Dr. F. E. Chidester and Mr. T. B. Hayne, jr., then associated with the writer in malaria-control work, collected Anopheles larvæ from the edge of a tidal marsh, in water showing a salinity of 10,048. Two specimens of *A. quadrimaculatus* emerged from this collection. Chidester and Hayne are both careful observers, and they expressed the opinion that this was a "complete" breeding place. However, in surveys extending over several seasons, under various conditions and in many salt-marsh areas, the writer has been unable to substantiate their findings.

(5) Experimental: Fourteen *A. quadrimaculatus* larvæ of all sizes were collected from a fresh-water pond and put into sea water, salinity 10,160. These were all dead within 12 hours, the larger ones surviving the longest. No mortality was noted in the pond-water controls.

Anopheles punctipennis.—The writer has never found breeding of *A. punctipennis* in salt or brackish water, nor is he aware of any report of such breeding. But there is much evidence that *A. punctipennis* has a wider range of breeding habits in fresh water than either *A. quadrimaculatus* or *A. crucians.* It is found in the coldest mountain springs and branches, and at times in streams and ditches foul with sewage. It is not resistant, however, to sea water.

Experimental: Twenty-five *A. punctipennis* larvæ of all sizes, collected from a ditch and a fresh-water lake, were transferred to sea water, salinity 10,160, and all were dead within seven hours. No mortality was noted in fresh-water controls.

Various experiments have been conducted by investigators to determine the value of salt as a larvicide. The following accounts are typical of the results obtained.

Veazie (1905) reports an attempt, during the outbreak of yellow fever in New Orleans, to destroy mosquito larvæ in the open gutters of the city by the use of common salt. The results were good where the work was properly done. Shortly after the operations were begun, there was a flight of *Aëdes sollicitans* from the salt marshes northeast of New Orleans. Indignant citizens, ascertaining from experts the name and habits of the species, jumped to the conclusion that the salting of the ditches had brought about suitable breeding conditions for *sollicitans* and that the invasion of the city by that species was a direct result of the work by the sanitary officials.

Peryassu (1908), with other workers in Brazil, made a series of experiments to determine the degree of salinity in which the larvæ of *A. argyrotarsis* could develop to imagoes. They found that in slightly brackish water imagoes were produced in a normal manner. In a mixture of 19 per cent of sea water with fresh water only a very small proportion of larvæ developed to imagoes. Beyond this the larvæ failed to pupate. With 20 per cent sea water some of the larvæ survived three days; with 30 per cent all died after one day.

Graham (1910) recommends the salting of water containing the larvæ of *Pyretophorus costalis*. He adds common salt in the proportion of 3 per cent and finds that it causes disintegration and precipitation of the motile algæ upon which the larvæ feed. The latter being thus deprived of their natural food, become cannibalistic. Salt, he says, in lesser concentration appears to inhibit the growth of young larvæ, probably by diminishing their food supply, but seems to hasten the fully grown larvæ, which become pupæ more rapidly than usual.

Darling (1910), working with the mosquitoes of the Canal Zone, observed the effect of salt and sea water on Anopheles larvæ and says: "In general, the effect of an irritating, toxic, or otherwise unusual fluid on mosquito larvæ is to hasten pupation. A number of experiments were tried with sea water, salt water, and solutions of the heavy metals, and in most instances, in the more concentrated solutions, when the larvæ were not killed within 24 hours, they pupated, and occasionally the period of pupation was shortened; so that if, for instance, sea-water were used as a larvicide, the first effect would be to hasten pupation, and thus increase the number of anophelines in a district, and if later the sea water became diluted by rain, several species of malaria-transmitting anophelines might breed in it without difficulty, notably *A. albimanus* and *A. tarsimaculata*. On this account sea water could not be used with any degree of success as a larvicide for anophelines, except in large quantities and in certain locations."

According to Howard, Dyar, and Knab, the proposed destruction of Anopheles by the introduction of sea water does not seem to be rational, at least with certain species. At all events the specific identity of the Anopheles concerned must be taken into account. It appears certain that while some species may breed either in fresh or brackish waters, others may occur exclusively in saline water, and still others only in fresh water.

CONCLUSIONS.

1. *A. crucians* was found to propagate in sea water diluted to a maximum salinity of 10,088 or slightly more than 50 per cent average sea water. The transfer of *A. crucians* larvæ, which had started their developemnt in brackish water, to sea water did not unfavorably affect their subsequent development.

2. *A. quadrimaculatus* was not found to breed in numbers sufficient to be of sanitary importance in a higher salinity than 10,003, or 1.5 per cent sea water. In one case two larvæ found in water with a salinity of 10,048 developed into *A. quadrimaculatus* imagoes, but this observation requires confirmation as to whether this species may complete its entire water cycle in so high a percentage of sea water. The question is raised as to whether *A. quadrimaculatus* larvæ may not withstand a much higher salinity intermittently than continuously. Specimens of *A. quadrimaculatus* transferred from fresh pond water to sea water, salinity 10,160, were all killed within 12 hours.

3. *A. punctipennis* was not found developing in salt or brackish waters. This species breeds under a wider range of conditions than either *A. quadrimaculatus* or *A. crucians*, but apparently does not survive in salt or brackish waters. Larvæ of *A. punctipennis* all died within seven hours when put into sea-water.

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NATIONAL HOSPITAL DAY.

The suggestion that a National Hospital Day be established and celebrated annually on May 12, the birthday of Florence Nightingale, pioneer in modern hospital and nursing methods, has swept the country since it was first suggested, 40 States and 4 Canadian Provinces having organized for it. President Harding has warmly approved the suggestion, and Surg. Gen. Cumming has written the following letter in regard to it to M. O. Foley, who originated the idea:

> I heartily approve the suggestion that May 12 be designated annual National Hospital Day, on which special efforts shall be made to diffuse information concerning hospitals.
>
> The public naturally lacks information on many points in regard to hospitals. For instance, although everyone who has tried to rent a house or who reads a daily paper knows that there is a marked shortage in buildings, few people realize that this shortage is particularly marked both in hospitals and in buildings that can be converted into hospitals. Most people, indeed, think that nearly any building can be made over into a hospital.